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July 24, 1997

The Secretary
Federal Communications Commission
1919 M. Street N.W. Room 222
Washington, DC 20554

In the Matter of	)	ET-Docket No. 93-62
	)	and in this docket pertaining to:
Guidelines for Evaluating the Environmental	)	- Report and Order FCC 96-326
Effects of Radiofrequency Radiation	)	- First Memorandum of Understanding Order FCC 96-487

Ex Parte Comments Pertaining to ET-Docket 93-62 Regarding
PETITIONS FOR RECONSIDERATION of Commission Rule & Order FCC 96-326, and First Memorandum of Opinion and Order FCC 96-487

with original and 1 copy submitted to the Secretary of the Commission
in accordance with 47 CFR Sections 1.1202, 1.1203, and 1.1206(a)
6th Ex Parte Submission

Dear Mr. Secretary,

Enclosed please find an original and 1 copy of an ex parte presentation pertaining to ET-Docket 93-62. Please assure these are put in the official record of this proceeding. The presentation includes the accompanying letter to the Commission and enclosed Exhibits #140B, #167-#187.

Thank you,

David Fichtenberg

David Fichtenberg
Ad-hoc Association of Parties Concerned About the Federal Communications Commission's Radiofrequency Health and Safety Rules
PO Box 7577
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July 24, 1997

Copy of presentation sent to: Chairman Reed E. Hundt
Federal Communications Commission
1919 M Street, N.W. Room 814
Washington, D.C. 20554

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Before the

FEDERAL COMMUNICATIONS COMMISSION

Washington, DC 20554

In the Matter of	)	ET-Docket No. 93-62
	)	and in this docket pertaining to:
Guidelines for Evaluating the Environmental	)	- Report and Order FCC 96-326
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To: The Commission

Ex Parte Comments Pertaining to ET-Docket 93-62
Regarding
PETITIONS FOR RECONSIDERATION of Commission Rule & Order FCC 96-326, and First Memorandum of Opinion and Order FCC 96-487

with original and 1 copy dated July 24, 1997 and submitted to the Secretary of the Commission in accordance with ex parte submission rules in 47 CFR Section 1.1202, 1.1203, and 1.1206(a)

Submitted by the Ad-hoc Association of Parties Concerned About the Federal Communications Commission's Radiofrequency Health and Safety Rules, PO Box 7577, Olympia, WA 98507-7577

6th Ex Parte Submission

1. Introduction:

1.1 Appropriate submission of an ex parte presentation

The Ad-hoc Association of Parties Concerned About the Federal Communications Commission's Radiofrequency ("RF") Health and Safety Rules ("the Ad-Hoc Association") understands (i) that a Federal Communications Commission ("Commission") "Sunshine Agenda" period per 47 CFR Section 1.1202(f) and Section 1.1203 is not now in effect regarding ET-Docket 93-62; (ii) that administrative finality has not yet been decided upon concerning the Commission's responses to Petitions For Reconsideration that have been submitted in this proceeding; and that (iii) this proceeding permits ex parte presentations in accordance with 47 CFR □1.1202, 1.1203, and 1.1206(a), and in accordance with the April 8, 1993 Notice of Proposed Rule Making in ET-Docket 93-62, paragraph 30. Accordingly, the Ad-Hoc Association is properly making this ex parte submission.

1.2. The primary purpose of this submission is to provide documentation of original sources to assist in and to facilitate the verifying of claims and evaluating of requests in petitions for reconsideration made by the Ad-Hoc Association or other parties concerned that the Commission's rules in this proceeding may not be sufficiently protective of the public health and who have submitted petitions for reconsideration of FCC 96-326 and FCC 96-487.

To the extent that these source documents were not previously referenced in presentations to the Commission, these documents and reports became available and understood after the last opportunity for filing in this matter, and in any event, consideration of these documents significantly provides support to claims of changes needed for the public health and their consideration is in the public interest.

In this way, the Ad-Hoc Association is providing an opportunity for the Commission to review and pass upon the matters presented herein, and by so doing the Commission will have the opportunity of verifying claims which have been made and of considering any newly discovered evidence which support the requests in the Ad-Hoc Association FCC 96-326 and FCC 96-487 petitions, and in any event, even if the Commission finds otherwise, the Commission's consideration of these documents which verify and further support the Commission's approval of Ad-Hoc Association requests is in the public interest.

Should the Commission find it should make changes elsewhere in its rules based on the evidence herein, it is requested that it do so, and make any other modifications it finds to be just and proper to serve the public interest.

2. Documents presented may help expedite the Commission asking the federal health agencies to which the Commission has chosen to defer for advice on RF safety matters - noting that the Ad-Hoc Association has requested the Commission be consistent in its policy of seeking such advice.

2.1 This documentation is provided to the Chairman of the Commission in order to provide these documents to those to whom the Commission defers for guidance in evaluating the claims and requests made in petitions for reconsideration by the Ad-Hoc Association and other parties concerned that which the Commission's rules may not be sufficiently protective - thereby facilitating such evaluations. In addition, while the Commission may not be expert in RF safety

matters, it may nevertheless review these documents and see evidence which would appear to raise significant questions about claims of safety made by the Commission and which appear to support the claims and requests of the Ad-Hoc Association. Since the Commission has stated its policy is for its rules to be based upon recent scientific findings, therefore it is requested the Commission be consistent in its policies and have these documents and claims and requests of the Ad-Hoc Association critically reviewed by the federal health agencies from whom the Commission has sought guidance in developing its RF safety rules. This is because, as noted before in these proceedings, the Ad-Hoc Association believes in advising the Commission the federal health agencies have overlooked or misunderstood important findings or there is new information which will likely change the recommendations that the federal health agencies provided to the Commission. For these reasons, the enclosed documents should be reviewed by the Commission and critically reviewed and evaluated by the federal health agencies with regard to the extent these documents provide sufficient levels of evidence, if not conclusive proof, that provide important support to the claims and requests of the Ad-Hoc Association.

2.2 Enclosed please find Exhibits numbered #140B, #167-#187 which are provided to the Commission in accordance with #1.1, 1.2, and #2.1 above and are submitted per "fair use" provisions of copyright law, and to be prudent should be presumed copyrighted materials unless stated or determined otherwise.

Respectfully Submitted,

David Fichtenberg

David Fichtenberg Dated: July 24, 1997
Spokesperson for the Ad-Hoc Association of Parties Concerned About the Federal Communications Commission's Radiofrequency Health and Safety Rules et al
PO Box 7577
Olympia, WA 98507-7577 Tel: (206) 722-8306

Enclosures: Exhibits numbered #140B, #167-#187

Author: IEEE Instrumentation and Measurement Technology Conference (1988 : San Diego, Calif.).
Title: Conference record : IEEE Instrumentation and Measurement Technology Conference, April 20-22, 1988, San Diego Princess Hotel, San Diego, California.
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E140B

ADVANCES IN DOSIMETRY OF RADIOFREQUENCY RADIATION AND THEIR PAST AND PROJECTED IMPACT ON THE SAFETY STANDARDS

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Abstract

The paper summarizes some of the highlights of the advances in the dosimetry of radio-frequency (RF) radiation that played an important role in the revision of the ANSI RF safety guidelines in 1982. Recent work has pointed to several potential problems with these guidelines. These pertain to large RF-induced currents and the commensurately high local SARs for some regions of the body, and contact hazards for commonly-encountered ungrounded objects in ANSI-recommended E-fields for the frequency band 0.3 - 100 MHz. The new data are used to suggest modifications of the RF safety guidelines.

Introduction

The expanding usage of electromagnetic (EM) radiation has necessitated an understanding of its interaction with humans. Such knowledge is vital in evaluating and establishing radiation safety standards, determining definitive hazard levels, and understanding several of the biological effects that have been reported in the literature. Studies of the effects of EM radiation have used laboratory animals such as rats, rabbits, etc. for the study of biological and/or behavioral effects. For these experiments to have any projected meanings for humans, it is necessary to be able to quantify the whole-body power absorption and its distribution for the various irradiation conditions. It is further necessary that dosimetric information be known for humans subjected to irradiation at different frequencies and for realistic exposure conditions.

Unlike ionizing radiation, where the absorption cross section of the biological target is directly related to its physical cross section, the whole-body EM energy absorption has been shown to depend stroggly on polarization (orientation of electric field E of the incident waves), frequency, and physical environments such as the presence of ground and other reflecting surfaces. A couple of excellent review articles [1,2] summarize the highlights of this work. Some of the most important findings are:

A. Maximum energy is absorbed for incident electric field along the height of the human body for frequencies such that the height h is approximately 0.36 to 0.4 times the wavelength (λ) for free space irradiation conditions. For a 1.75 m-tall individual this correspondes to a frequency on the order of 65 to 70 MHz. Power absorbed under these conditions is 4.2 times larger than that projected from physical cross section considerations. This is the resonant region of absorption of EM radiation [3].

B. For frequencies below resonance, an f^2-type dependence has been observed for the energy absorbed, where f is the frequency of radiation. For the post-resonant region, the absorbed power reduces as 1/f, approaching gradually the value that would correspond to roughly one-half that based on physical cross-sectional considerations.

C. For a human standing on a high conductivity ground, the frequency for maximum absorption is approximately one-half that without the ground; i.e., about 35 MHz. At the new resonant frequency, the power absorbed is somewhat larger and corresponds to roughly 8 times that projected from physical cross-section considerations [4,5,6].

The above results have had an impact on the 1982 reformulation of the American National Standards Institute (ANSI) guideline [7] as well as the guidelines [8] formulated by the International Nonionizing Radiation Committee of the International Radiation Protection Association (IRPA). The ANSI-1982 guideline for safety levels with respect to human exposure to radiofrequency (RF) electromagnetic fields, 300 KHz to 100 GHz, is shown in Fig. 1. The protection guide recommends as safe a power density level of 100 mW/cm^2 (electric field E ~ 614 V/m,) for the frequency region 0.3 to 3.0 MHz, with a safety level reducing as $900/f^2_{MHz}$ mW/cm^2 ($E \sim 1842/f_{MHz}$ V/m, f is the frequency in MHz) for the frequency region 3.0 to 30.0 MHz to a valley of power density P = 1 mW/cm^2 (E ~ 61.4 V/m) for the frequency band 30 to 300 MHz. The ANSI guide has been prescribed to ensure that the whole-body-averaged SAR shall not exceed 0.4 W/kg for any of the human sizes and age groups. Dosimetric information on e.m. energy absorption in human beings [1-3] was used to obtain the power density as a function of frequency so that under the worst-case circumstances (E-field along the height of the body; grounded and ungrounded conditions), the whole-body-averaged SAR will be less than 0.4 W/kg. Recognizing the highly nonuniform nature of SAR distribution including some regions where there may be fairly high local SARs, the ANSI guide further prescribes that the local SAR "in any 1-g of tissue" shall not exceed 8 W/kg. A recent report [9] by National Council on Radiation Protection (NCRP) has recommended lower power densities that are one-fifth of the values given in the ANSI guideline [7] for public exposures.

Recent studies have pointed to several problems with the ANSI RF safety guideline, particularly in the VLF to HF range of frequencies. These are highlighted in the following:

A. The high electric fields sanctioned in the ANSI guideline for LF to HF frequencies will result in significant RF currents flowing through the human body resulting in high SARs in smaller cross section areas of the body such as the leg and the ankle region [10,11]. Based on measurements with standing human subjects for plane-wave fields, induced currents on the order of 628-780 mA and resulting ankle section SARs as high as 182-243 W/kg are projected for 1.75 m-tall individuals for the ANSI guideline for the frequency band 3-40 MHz. Using electromagnetic scaling concepts, SARs as high as 371 and 534 W/kg are projected for ten- and five-year-old children, respectively, for f = 50.7 and 62.5 MHz. These values are considerably larger than 8 W/kg for "any 1-g of tissue" assumed in the ANSI guideline.

B. Commonly encountered ungrounded objects such as car, van, bus, etc. will develop open circuit voltages on the order of several hundred volts exposed

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to ANSI-recommended electric field of 614 V/m for the frequency band 0.3 to 3 MHz. Upon touching such vehicles large currents may flow through the human body that are considerably in excess of those needed for perception, pain and even burns in some cases [6,12,13]. For example, the current flowing through the hand of a human upon holding the door handle of an ungrounded automotive van is estimated to be 879 mA resulting in a local SAR in the wrist of about 1045 W/kg.

To reduce the abovementioned problems, new limits have recently been proposed by IRPA and by Dr. Maria Stuchly of Health & Welfare, Canada [14, 15]. Using the recent data [6,10-13], we propose the following modifications of the radio-frequency protection guides to limit the RF currents that can be induced in the human body.

Suggested Radio-frequency Protection Guide (RFPG) for Occupational Exposures

The proposed RFPG is given in Table 1 and plotted in Fig. 2.

Table 1. Proposed radio-frequency protection guides for occupational exposures.

Frequency Range (MHz)	E V/m	H A/m	Plane-Wave Equivalent Power Density (mW/cm^2)
0.003 - 0.1	* 614	163	—
0.1 - 3.0	* 614	16.3/f	—
3 - 30	* 1842/f	16.3/f	—
30 - 100	* 61.4	16.3/f	—
100 - 300	61.4	0.163	1.0
300 - 3000	$61.40 \times (f/300)^{1/2}$	$0.163 \times (f/300)^{1/2}$	f/300
3000 - 300,000	194	0.5	10.0

Note: f = frequency in MHz. E and H are the magnitudes of electric and magnetic fields, respectively.

* The personnel access areas should be restricted to limit induced RF body currents and potential for RF shock and burns, as defined in the following.

Since higher E-fields proposed in Table 1 for the band 0.003-1000 mHz, ff these were vertical, would result in high RF induced body currents and a potential for shock and burns for contact with ungrounded metallic bodies, the personnel access areas should be limited in the following manner:

1. For free-standing individuals (no contact with metallic bodies), RF current induced in the human body is less than or equal to 100 mA as measured through both feet or 50 mA through each of the feet. For a frequency of less than 100 kHz, the allowable induced current should be reduced as follows:

$$I = 1.0\ f_{kHz} \quad mA \qquad (1)$$

The above limitations on RF induced currents are suggested to ensure that the ankle-section SARs for frequencies higher than 0.1 MHz will be no more than 5.8-10.7 W/kg for adults of heights 1.75-1.5 m. For frequencies lower than 100 kHz, the current densities in the ankle section will be slightly lower than those needed for stimulating thresholds for the nerve/muscle system [16].

For vertically polarized electric fields, the above limitation on current would imply [11,17] that the permissible E-field is less than (300/f) V/m for frequencies in excess of 0.1 MHz.

2. For conditions of contact with metallic bodies, maximum RF current through an impedance equivalent to that of the human body for conditions of grasping contact (see ref. 13, Fig. 1), as measured with a contact current meter shall not exceed the following values:

$$I = 0.5\ f_{kHz} \quad mA \quad \text{for } 3 < f < 100 \text{ kHz} \qquad (2)$$

$$= 50 \text{ mA} \quad \text{for } f > 0.1 \text{ MHz} \qquad (3)$$

The current limits given by Eqs. 2 and 3 would help ensure that the current experienced by a human being upon contacting these metallic bodies would be less than that needed for perception or pain at each of the frequencies [13].

Steps such as grounding and use of safety equipment that result in reduced currents would obviously allow existence of higher fields without exceeding the above limits for conditions of contact with metallic bodies.

Significantly higher RF magnetic fields are recommended in the proposed RFPG of Table 1. For the frequency band 0.1-100 MHz, the RF magnetic field guideline is

$$H = \frac{16.3}{f} \quad A/m \qquad (4)$$

For magnetic fields given by Eq. 4, the peak and whole-body-averaged SARs have been calculated using an anatomically-realistic model of an adult human being [18]. These are given in Table 2 along with the peak internal current densities. A magnetic-field orientation from front to back of the body is assumed for these calculations. This orientation was selected because of its strongest coupling to the human body.

For frequencies less than 0.1 MHz, an RF magnetic field of 163 A/m implies a peak current density $< 0.0117\ f_{kHz}$ mA/cm^2 which is considerably lower than the threshold of perception of currents at these frequencies [13, 16].

Suggested RFPG for the General Public

The proposed RFPG for the general public is given in Table 3. This RFPG is plotted in Fig. 3. The explanations for superscripts a - d in Table 3 is given in the following:

(a) The electric field E suggested for these frequency bands is lower than the threshold of perception of commonly encountered metallic bodies such as a car, a van, etc. It is, however, close to the threshold of perception for finger contact of a school bus by a child [13].
(b) For the E-field suggested here, the current induced in a free-standing (no contact with metallic bodies) human being is less than or equal to 100 mA (one leg current = 50 mA), which is consistent with the access area limitation for occupational exposures.
(c) An incident electric field of 8 V/m implies the

numbers given in Table 4 for maximum induced currents and ankle-section SARs.

(d) We have previously projected that whole-body-exposure millimeter-wave power densities on the order of 8.7 mW/cm^2 are likely to cause sensations of "very warm to hot" [20]. At higher frequencies, a power density of 1 mW/cm^2 is suggested to prevent threshold of perception of warmth. Also the suggested power density of 1 mW/cm^2 is consistent with the recently proposed NCRP guideline for the general population [9].

Table 2. Whole-body-averaged and peak SARs for an anatomically realistic model of an adult human being for RF magnetic fields given by Eq. 4. A magnetic field orientation from front to back of the body is assumed to obtain highest possible SARs.

f_{MHz}	H A/m	Whole-body-Averaged SAR W/kg	Peak Current Density mA/cm^2	Peak SAR W/kg
0.1	163*	0.014	1.17	0.31
0.3	54.3	0.013	1.17	0.29
1.0	16.3	0.012	1.17	0.27
3.0	5.43	0.011	1.17	0.24
10.0	1.63	0.010	1.17	0.22
30.0	0.54	0.009	1.17	0.20
100.0	0.16	0.006	1.17	0.13

* A magnetic field of 163 A/m corresponds to a magnetic flux of 2.05 Gauss in the Gaussian system of units.

Table 3. Proposed radio-frequency protection guides for general population.

Frequency Range MHz		E** V/m	H** A/m	Plane-wave Equivalent Power Density mW/cm^2
0.003 - 0.1	(a)	61.4*	163	——
0.1 - 1.0	(a)	61.4*	16.3/f	——
1.0 - 3.9	(b)	61.4†	16.3/f	——
3.9 - 100	(c)	240/f†	16.3/f	——
30 - 100		8†	16.3/f	——
100 - 5,900		$0.8f^{1/2}$†	0.163	(d) ——
5,900 - 300,000		61.4	0.163	1.0

* spatially-averaged over a volume corresponding to that of an automobile.

† spatially-averaged over a volume corresponding to that of a human being.

** The frequency f is in MHz. E and H are the magnitudes of electric and magnetic fields, respectively. Explanation for (a)-(d) is given in the text.

Comparison of the Recommended RFPGs with Standards at Other Frequencies

From Eq. 2, the suggested limit on the contact current is 1.5 mA at 3 kHz. This may be compared with the National Electric Safety Code [21] which specifies a maximum leakage current of 0.5 mA from portable electrical tools and household applicances and 0.75 mA for permanently-fixed appliances. Recognizing the the threshold current for perception at 3 kHz is approximately 3 times higher than that at 60 Hz [22], a suggested contact current of 1.5 mA is not out of line with the leakage current of 0.5-0.75 mA specified in the National Electric Safety Code.

Table 4. Induced currents and ankle-section SARs for an incident E-field of 8 V/m [11].

	Height m	Frequency MHz	I_h mA	Ankle SAR* W/kg
Average adult	1.75	40.0	101.6	3.8
Adult	1.5	46.7	87.2	5.0
10-year-old child	1.38	50.7	80.1	8.7
5-year-old child	1.12	62.5	65.0	7.8

* We have assumed a conductivity of 0.693 S/m for the high-water content tissues at 40 and 46.7 MHz [19]. Somewhat larger conductivities of 0.73 and 0.77 S/m are taken at 50.7 and 62.5 MHz, respectively. The corresponding dielectric constants taken are 97.3 for 40 and 46.7 MHz and 92.9 and 87.4 for 50.7 and 62.5 MHz, respectively.

For higher RF frequencies, the suggested guideline of 10 mW/cm^2 for occupational exposures is in agreement with the occupational standard for infrared radiation, while a reduced guideline of 1 mW/cm^2 (see Table 3) is consistent with the recently proposed NCRP guideline [9] for the general population.

Concluding Remarks

The proposed radio-frequency protection guides depart from the previous guidelines such as those by ANSI [7] in two respects:

1. An increase in the safety levels of RF magnetic fields for frequencies less than 100 MHz. This is proposed in recognition of the fact that these fields do not couple as tightly as do the E-fields and do not cause substantial SARs.

2. Limitation on the induced currents in the human body to no more than 100 mA for frequencies in excess of 100 kHz and a linearly reducing current for lower frequencies. The current under contact situations is limited to 50 mA for frequencies in excess of 100 kHz and a proportionally reducing current at lower frequencies.

Identical current limitations are proposed for both occupational and public exposures. Since safety measures can be adapted in the work place, higher field limits are suggested provided steps are taken to limit the currents for contact as well as for noncontact situations. Procedures are indeed available to measure the foot currents through the human body using shoe-mounted RF current sensors [23]. Methods similar to those of Fig. 1, reference 13, can be used to estimate RF currents through the human body for conditions of contact with metallic bodies. For these measurements an impedance equivalent to that of the human body for conditions of grasping contact may be used.

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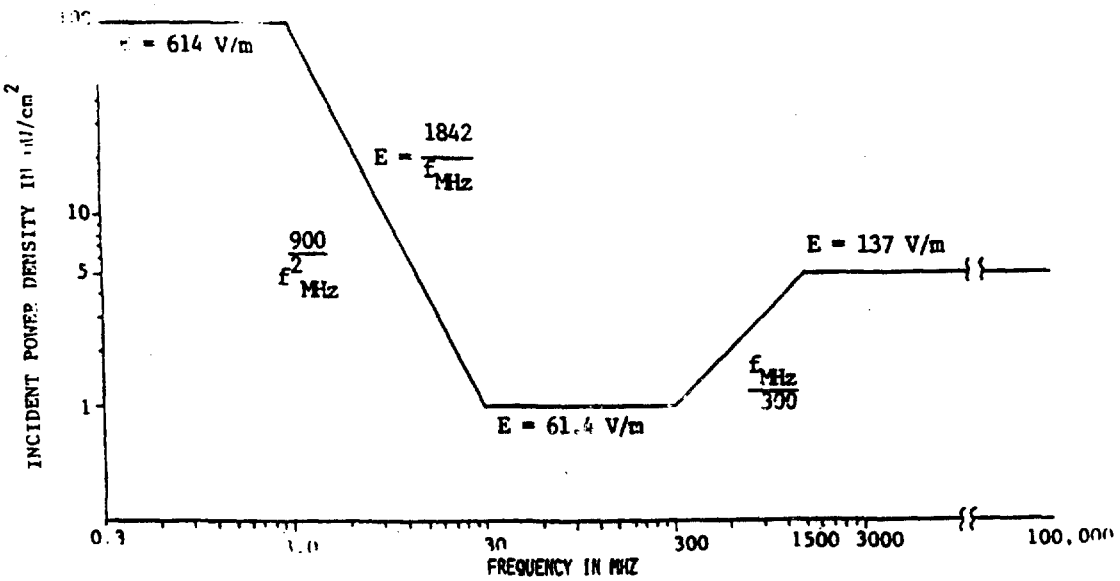

Fig. 1. ANSI C95.1-198[illegible] safety guide for human exposure to RF electromagnetic fields [Ref. 7].

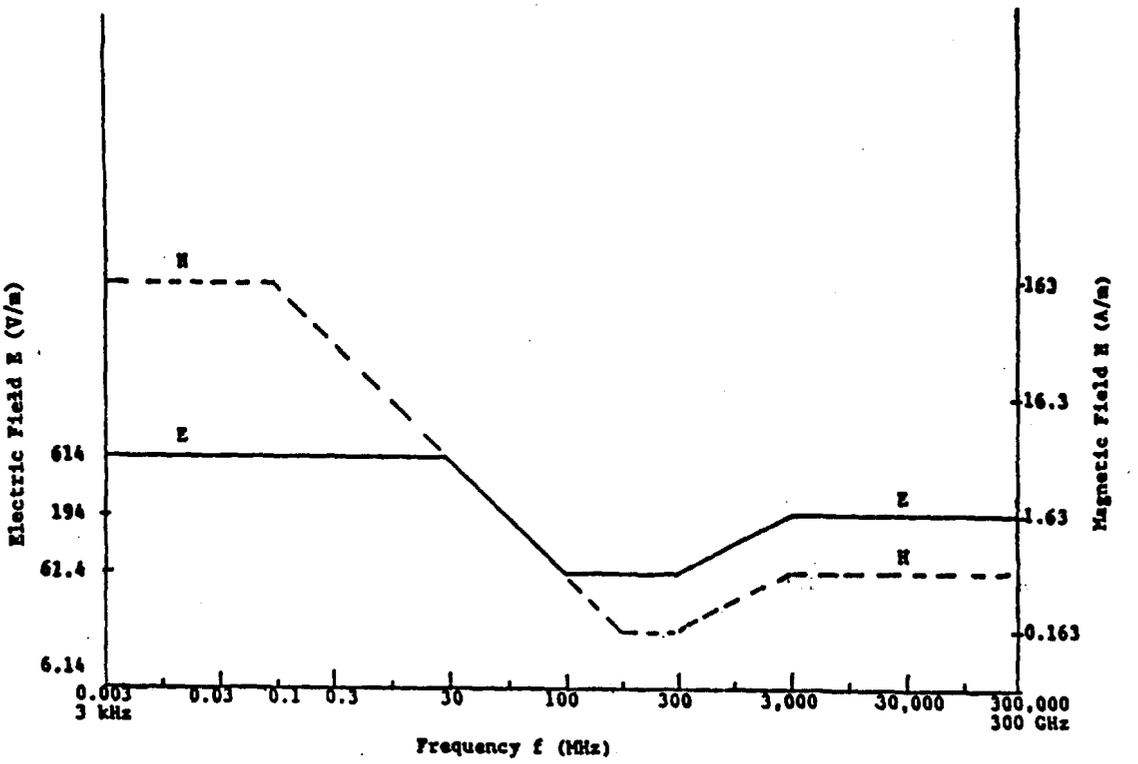

Fig. 2. Proposed radio-frequency protection guide for occupational exposures.

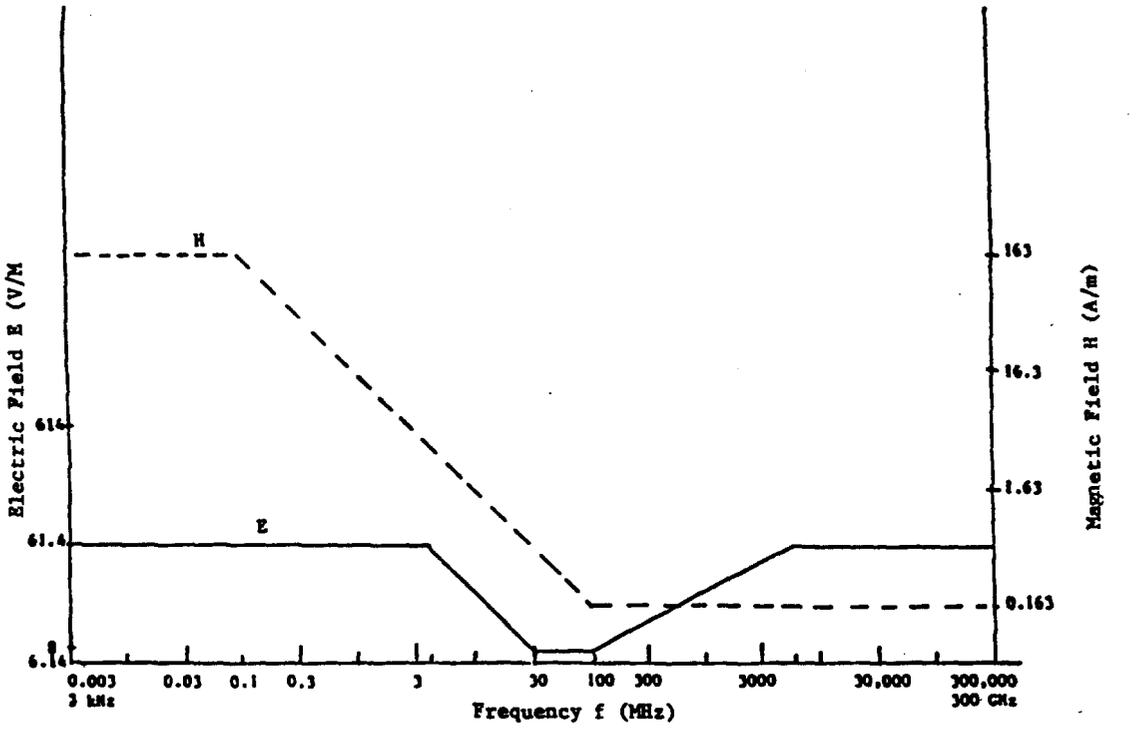

Fig. 3. Proposed radio-frequency protection guide for the general population.

E167

Bioelectromagnetics 17:312–321 (1996)

Resonance Effect of Millimeter Waves in the Power Range From 10^{-19} to 3×10^{-3} W/cm^2 on *Escherichia coli* Cells at Different Concentrations

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The effect of millimeter waves (MMWs) on the genome conformational state (GCS) of *E. coli* AB1157 cells was studied by the method of anomalous viscosity time dependencies (AVTD) in the frequency range of 51.64–51.85 GHz. The 51.755 GHz resonance frequency of the cell reaction to MMWs did not depend on power density (PD) in the range from 10^{-19} to 3×10^{-3} W/cm^2. The half-width of the resonant reaction of cells showed a sigmoid dependence on PD, changing from 3 MHz to 100 MHz. The PD dependence of the half-width had the same shape for different concentrations of exposed cells (4×10^7 and 4×10^8 cells/ml), whereas the magnitude of the 51.755 GHz resonance effect differed significantly and depended on the PD of MMW exposure. Sharp narrowing of the 51.755 GHz resonance in the PD range from 10^{-4} to 10^{-7} W/cm^2 was followed by an emergence of new resonance frequencies. The PD dependence of the MMW effect at one of these resonance frequencies (51.674 GHz) differed markedly from the corresponding dependence at the 51.755 GHz resonance, the power window occurring in the range from 10^{-16} to 10^{-8} W/cm^2. The results obtained were explained in the framework of a model of electron-conformational interactions. The frequency-time parameters of this model appeared to be in good agreement with experimental data.

Key words: low-intensity microwaves, genome conformation, viscosity, electron-conformational interaction model, half-width of resonance

INTRODUCTION

The biological effect of millimeter waves (MMWs) or electromagnetic fields (EMFs) of extremely high frequency has been discussed for more than 20 years [Webb and Booth, 1971; Vilenskaya et al., 1972; Devyatkov, 1973; Gründler et al., 1988]. However, the mechanism of these effects has yet to be elucidated. Moreover, some biological effects of MMWs have been questioned due to poor reproducibility [Motzkin et al., 1983; Gandhi, 1983]. One reason for poor reproducibility may be that the effects of low-intensity MMWs are dependent on numerous genetic, physiological, and physical parameters that are not always reproduced in different laboratories. For instance, the MMW effect on *Escherichia coli* cells is dependent on the growth stage of the bacterial culture [Belyaev et al., 1993a], on the cell concentration and the magnitude of the static magnetic field during exposure [Belyaev et al., 1994a], on the MMW polarization [Belyaev et al., 1992b,c], and on the time between microwave exposure and registration of the effect [Belyaev et al., 1994a].

It has been previously established that nonthermal MMWs influenced the genome conformational state (GCS) of *E. coli* cells [Belyaev et al., 1992a] and thymocytes of rats [Belyaev and Kravchenko, 1994]. This effect was dependent on the frequency and polarization of the MMWs. To account for these data, it has been proposed that a set of resonance frequencies is determined by a genome structure and that an effective polarization is determined by a helicity of DNA sequences that interact efficiently with MMWs [Belyaev et al., 1992b,c]. This assumption was further supported by the finding that the induction of approximately one single-strand DNA break per genome of *E. coli* cell by means of irradiation with X-rays eliminated the difference in biological effect of left- and right-handed circularly polarized MMW [Belyaev et al., 1992d].

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Two physical mechanisms have been proposed in support of the idea that the natural resonance frequencies of MMW interaction with living cells are determined by the genome structure. The first model describes the oscillations in the regulatory DNA sequences that control elementary genetic processes of transcription, replication, recombination, and repair [Arinichev et al., 1993]. The second model considers the natural mechanical and acoustic oscillations in the domains of supercoiling [Belyaev et al., 1993b]. This latter model is related to the model of direct excitations in DNA that was developed previously [Davis et al., 1986]. Based on these models, the relationship between the resonance frequencies, the mass of the nucleoid, and the genome length was obtained. The predicted theoretical dependence of resonance frequencies on the length of the genome was supported in experiments where the length of the bacterial genome was changed by inserting DNA of different prophages into an *E. coli* chromosome [Belyaev et al., 1993b].

If a resonance frequency is determined by the genome structure only, then it would be insensitive to power density (PD). In this study, we examined the PD dependence of the resonance frequency of a 10 min MMW effect within the range from 10^{-19} to 3×10^{-3} W/cm^2.

We have previously established that reduction of the PD from 10^{-4} to 10^{-14} W/cm^2 resulted in a significant narrowing of the resonance response of *E. coli* cells to MMW exposure [Belyaev et al., 1992d]. Similar results have been obtained with yeast cells [Gründler, 1992] and thymocytes of rats [Belyaev and Kravchenko, 1994]. In this study, we examined the PD dependence of resonance half-width down to the minimal value of 10^{-19} W/cm^2, where a MMW effect was previously observed [Belyaev et al., 1994a]. Because the magnitude of the resonant reaction of cells to MMWs appears to be dependent on the concentration of exposed cells [Belyaev et al., 1994a], the PD dependence of the resonant MMW effect on *E. coli* cells at different concentrations was also investigated. The MMW effects were examined by using the method of anomalous viscosity time dependencies (AVTD). A correlation of the AVTD measurements with the GCS changes was examined and discussed previously [Belyaev et al., 1992a,d, 1993a]. In this study, we provided new evidence for such correlation by using ethidium bromide, which is known as a specific intercalating drug for DNA [Cook and Brazell, 1976].

MATERIALS AND METHODS

E. coli K12 AB1157 cells were grown in 100 ml of Luria broth (Difco; tryptone 10 g/liter, yeast extract 5 g/liter, NaCl 10 g/liter) for 20 h, as previously described [Belyaev et al., 1993b]. Under these conditions, the cells reached a concentration of about 10^9 colony-forming units/ml and an optical density of 0.85–1.05, which corresponded to the early stationary phase of bacterial growth [Belyaev et al., 1993a].

The experimental unit for cell exposure to MMW (Fig. 1) has already been described [Belyaev et al., 1992a, 1994a]. Voltage standing wave ratio (VSWR) in the waveguide system was measured by means of a measuring instrument at 1 mW incident power. The VSWR did not exceed 1.4 in different points of the waveguide system. The reflected power was less than 10% at the studied frequency range. The MMW frequency was measured by using two frequency converters and a frequency meter. The frequency deviation was not more than 1 MHz. The output power was measured by a wattmeter in the range of from 6×10^{-7} to 6×10^{-2} W. All of the MMW devices were made in the former USSR. Average PD at the exposure site was calculated by dividing the output power by the projected area of the horn at the surface of the Petri dish. A PD of less than 10^{-7} W/cm^2 was obtained by means of four metallic flap attenuators, which were calibrated in the range from 6×10^{-7} to 6×10^{-2} W. Each of them reduced the MMW intensity by 40–50 dB. Attenuators were calibrated in the frequency range of 51.6–51.9 GHz. Additional adjustment of the PD was also carried out by changing the oscillator output level with a maximum attenuation of 30 dB. In some experiments, the last flap attenuator in the waveguide system was replaced by a carbon attenuator. This attenuator was made by inserting a carbon-containing absorber into the waveguide segment. It reduced the MMW intensity by 40 dB. We did not observe significant differences in results for the different types of attenuator.

The local distribution of PD on the surface of a styrofoam plate between the horn and the exposed Petri dish was measured at 100 μW/cm^2, as previously described [Belyaev et al., 1992a]. The maximum deviation of local PD on this surface did not exceed 5 dB. A frequency change of a few GHz could lead to a marked shift of PD minima and maxima, including changing an increase to a decrease or vice versa. But frequency changes of ±150 MHz around the 51.755 GHz frequency did not lead to significant changes in the pattern of PD distribution. The PD value, which was averaged over the whole surface under irradiation, did not change for different frequencies if the output level was the same.

Cells were exposed in Petri dishes (50 mm in diameter). Each dish contained 3.5 ml of a cell suspension. The suspension temperature was measured by a microthermocouple with a 0.1 °C accuracy. The average heating of the cell suspension did not depend on the MMW frequency in the frequency range under study. No heating was measurable when the PD was less than

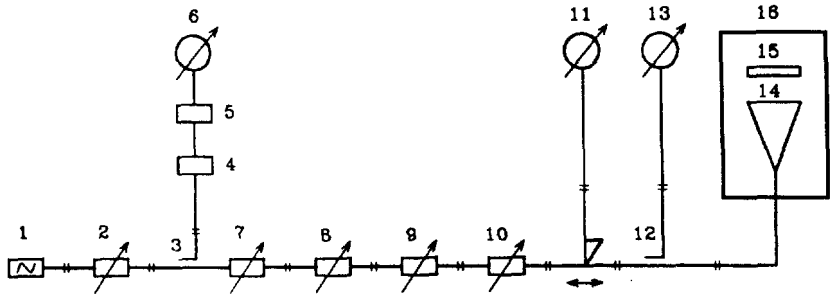

Fig. 1. Block diagram for the millimeter wave (MMW) irradiation of cells. 1, a backward wave tube of oscillator; 2, an internal attenuator of the oscillator; 3,12, directional couplers to separate about of 10% of the output power for measurements; 4,5, frequency converters; 6, frequency meter; 7–10, calibrated flap attenuators; 11, measuring instrument for the voltage standing wave ratio (VSWR) measurements in a waveguide system; 13, wattmeter with an accuracy of measurements better than 10%; 14, pyramidal horn with dimensions of 40 × 50 mm²; 15, Petri dish (50 mm in diameter) with a cell suspension; 16, chamber for irradiation.

10^{-4} W/cm². The Petri dishes were closed during exposure and the prelysis incubation. The cells were exposed in a special chamber that was shielded by carbon-containing tissue and aluminum foil. In each experiment, the sham exposure was carried out while the oscillator was working at the resonance frequency and while the MMW radiation was maximally attenuated by all attenuators down to PD < 10^{-21} W/cm². Our exposure system was clamped well to avoid mechanical vibrations.

Before exposure, the cells were diluted to a concentration of 4×10^7 or 4×10^8 cells/ml, as previously described [Belyaev et al., 1994a]. Incubation of cells after dilution was not less than 30 min. Cells were exposed in Petri dishes for 10 min and incubated for 120 min before lysis, as previously described [Belyaev et al., 1994a]. It has been shown previously that a weak change in the static magnetic field could affect the GCS of *E. coli* cells [Belyaev et al., 1994b]. In this study, we controlled the static magnetic field, as described previously [Belyaev et al., 1993a]. This field was 48 ± 5 μT in the places of growth, exposure, and incubation of cells.

The cells were lysed, and the GCS changes were measured in lysates by using the method of AVTD, as previously described [Belyaev et al., 1992a]. Briefly, this method is based on the radial migration of large DNA-protein complexes in the high-gradient hydrodynamic field of a rotary viscometer. Radial migration of molecular complexes toward the rotating rotor causes anomalous changes of viscosity that can be registered by measuring the rotor rotation period as a function of time. This AVTD depends strongly on the conformational state of the genome, which, in turn, is dependent on DNA parameters such as molecular weight, micromedium, and the number of proteins bound to the DNA. Each AVTD curve is a set of 30–50 experimental points (period of rotation vs. time of measurement) that were recorded by an IBM PC. The AVTDs were measured at a shear rate of 5.6 s^{-1} and at a shear stress of 0.007 N/m².

The maximum period of rotation (T_{max}) corresponds to maximum viscosity and has previously been shown to be the most sensitive AVTD parameter. The significance of differences between mean values in irradiated samples ($T_{max\ irr}$) and control samples ($T_{max\ con}$) was evaluated with Student's t test in each experiment. Maximum relative viscosity (η) was used to determine the MMW effect on the GCS: $\eta = (T_{max\ irr})/(T_{max\ con})$. Results were considered as significantly different at $P < .05$. Each version of the experiment included not less than three measurements, which were compared to corresponding variants of intact and sham-exposed cells by using Student's t test. Comparison of control with sham control revealed no significant differences. We performed some experiments blind. An independent observer analyzed the data without any knowledge of which sets of samples were exposed. Both kinds of experiments, blind and ordinary, resulted in the same data.

RESULTS

Figure 2 presents three frequency dependencies of the MMW effect on the GCS of *E. coli* AB1157 cells that were exposed at a concentration of 4×10^7 cells/ml. In summary, 19 experiments were performed. In these experiments, the PD changed in the range from 10^{-19} W/cm² to 3×10^{-3} W/cm². In each experiment, the frequency dependence of the effect showed a resonance nature and fitted well to a Gaussian distribution with a significance level of $P < 5 \times 10^{-4}$ to .05. The center frequency of the resonance did not depend on PD and was 51.755 ± .001 GHz (Table 1). A study of the MMW effect on cells that were exposed at a concentration of 4×10^8 cells/ml revealed similar regularities: 1) a statistically significant

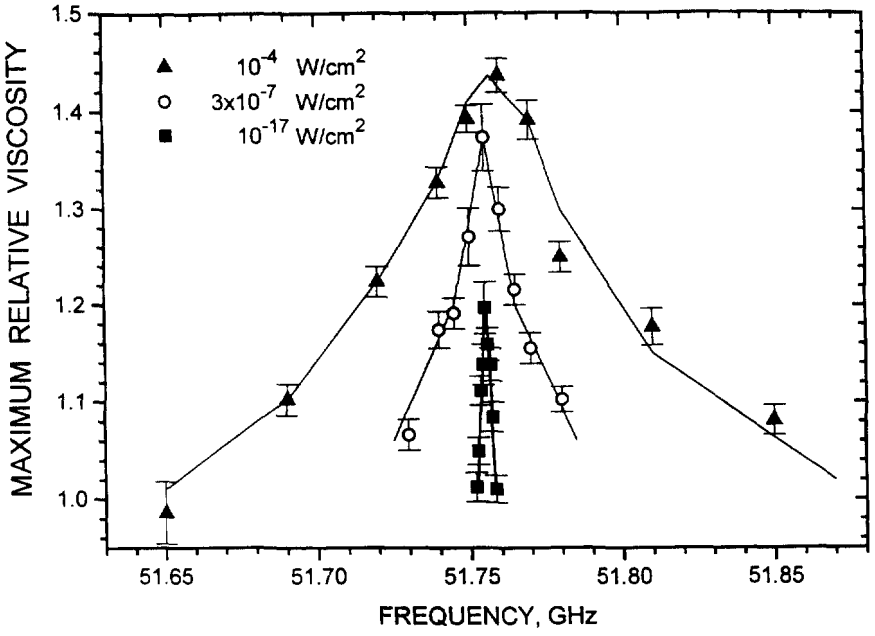

Fig. 2. Dependencies of maximal relative viscosity of cell lysates on frequency of cell exposure to MMWs at different power densities (10^{-4} W/cm^2, 3×10^{-7} W/cm^2, and 10^{-17} W/cm^2).

TABLE 1. Resonance Frequencies of Millimeter Wave Effect on the Genome Conformational State of *E. coli* Cells at Different Power Densities and Cell Concentrations During Exposure*

	Cell concentration during exposure	
Power density (W/cm^2)	4×10^7 cells/ml	4×10^8 cells/ml
10^{-19}	—	51.755 ± .001
10^{-18}	51.755 ± .001	51.755 ± .001
10^{-18}	51.755 ± .001	51.755 ± .001
10^{-17}	51.755 ± .001	—
10^{-16}	51.755 ± .001	51.755 ± .001
10^{-15}	51.755 ± .001	—
10^{-14}	51.755 ± .002	—
10^{-13}	51.756 ± .002	—
10^{-10}	51.755 ± .002	51.755 ± .002
10^{-10}	51.754 ± .002	—
10^{-10}	51.753 ± .002	—
10^{-10}	51.76 ± .01	—
10^{-8}	51.755 ± .004	—
10^{-7}	51.756 ± .002	—
3×10^{-7}	51.755 ± .005	—
10^{-6}	51.756 ± .002	51.755 ± .005
10^{-5}	51.755 ± .005	51.755 ± .005
10^{-4}	51.76 ± .01	51.76 ± .02
5×10^{-4}	51.76 ± .01	51.76 ± .01
5×10^{-4}	—	51.76 ± .01
3×10^{-3}	51.76 ± .01	—

*The uncertainty in frequency shown reflects the steps used in changing frequency in each experiment.

approximation of frequency dependence by the Gaussian distribution and 2) stability of the 51.755 ± .001 GHz resonance frequency at different PDs.

Thus, the resonance frequency of 51.755 GHz did not depend on PD for both cell concentrations during exposure. At the same time, the half-width of the resonance (width at half-maximum of the effect) displayed a strong PD dependence in both cases. It can be seen in Figure 3 that this dependence had the same shape for both cell concentrations. Specific features of this dependence were: 1) very weak growth, which was close to a plateau of about 3–4 MHz in the range from 10^{-19} to 10^{-15} W/cm^2; 2) weak growth in the range from 10^{-15} to 10^{-7} W/cm^2; and 3) a sharp increase in half-width up to about 100 MHz in the range from 10^{-7} to 10^{-4} W/cm^2.

The minimum half-widths of the resonance MMW effect were determined in our experiments as 3.1 ± .1 MHz at PD = 10^{-18} W/cm^2 for the 4×10^7 cells/ml concentration during exposure and 3.0 ± .5 MHz at PD = 10^{-19} W/cm^2 for the 4×10^8 cell/ml concentration. Unlike the half-width of the resonance, the dependence of the magnitude of the resonance effect on PD showed a considerable difference from one concentration of exposed cells to another (Fig. 4). The relative MMW effect, in which each exposed culture was normalized to its own control at each concentration, was greater at higher concentrations of cells than at lower concentrations. At both concentrations, the PD dependence of the effect had a plateau region. This plateau was reached at quite

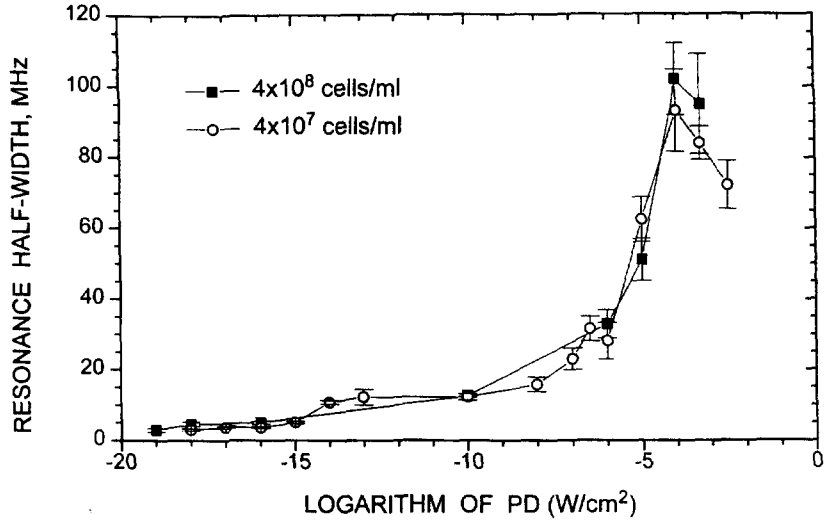

Fig. 3. Dependence of half-width of the 51.755 GHz resonance effect on power density of cell exposure at different concentrations (4×10^8 cells/ml and 4×10^7 cells/ml).

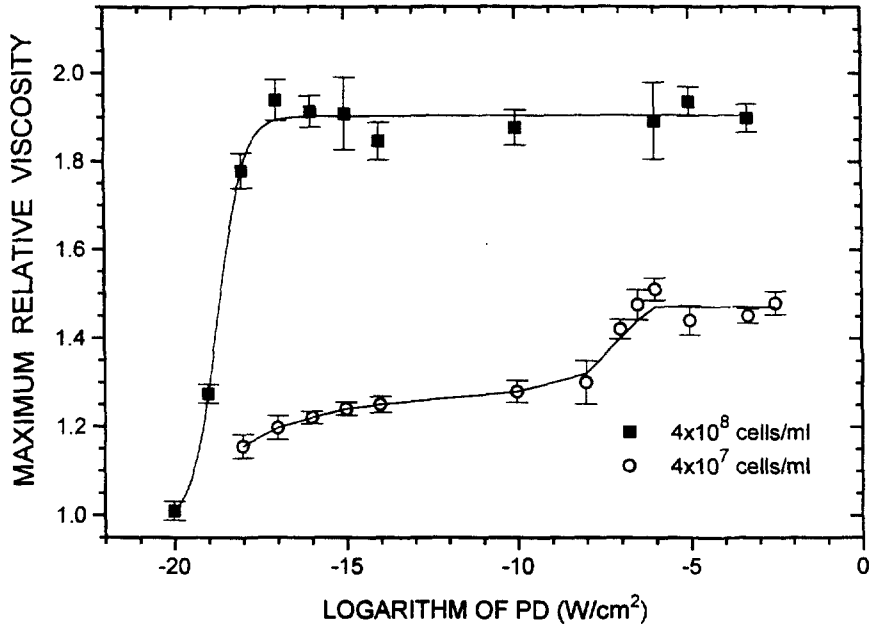

Fig. 4. Power density dependencies of maximal relative viscosity after exposure of *E. coli* cells to MMWs with 51.755 GHz frequency at different cell concentrations (4×10^8 cells/ml and 4×10^7 cells/ml). Lysis was performed at the concentration of 4×10^7 cells/ml.

different PDs: 1) 10^{-6} W/cm^2 if cells were exposed at a concentration of 4×10^7 cells/ml and 2) 10^{-17} W/cm^2 for a concentration of 4×10^8 cells/ml.

We studied the frequency dependence of the MMW effect at 10^{-10} W/cm^2 within the frequency range of 51.65–51.85 GHz. In this range, the resonance response with a half-width of about 100 MHz was observed at 10^{-4} to 10^{-3} W/cm^2. Four sharp resonances were detected at PD = 10^{-10} W/cm^2: 51.674 ± .003, 51.755 ± .001, 51.805 ± .002, and 51.835 ± .005 GHz (Fig. 5). Each resonance was reproduced in two to four independent experiments. Half-widths of these four

resonances were about 10 MHz and could not be distinguished within the error of measurements.

It was established in preliminary experiments that MMWs at 51.674 GHz and a PD of 3×10^{-3} to 10^{-4} W/cm^2 did not produce significant effects on the GCS of *E. coli* cells. At the same time, MMWs of the same frequency caused an approximately 30% increase in the AVTD peaks when cells were exposed at 10^{-10} W/cm^2 (Fig. 5). This dependence of the MMW effect on the power density was different from one that was obtained previously when exposing the cells to MMWs at the 51.755 GHz resonance frequency (Fig. 4). In this connection, we studied the dependence of MMW effect at the 51.674 GHz frequency on PD in more detail. This dependence, averaged over three independent experiments, had the shape of a "window" in the PD range from 10^{-16} to 10^{-8} W/cm^2 (Fig. 6). Such a dependence cannot be explained by a thermal effect of MMWs. We investigated the frequency dependence of effects in the frequency range of 51.664–51.688 GHz in five experiments. The 51.674 GHz resonance frequency was found to be independent of PD in the range from 10^{-18} to 10^{-8} W/cm^2 within the 3 MHz error. No fine structure in the resonance dependence of the MMW effect at 3×10^{-3} and 10^{-4} W/cm^2 PDs was detected in the frequency range of 51.805–51.830 GHz.

Figures 2–6 show that the exposure of cells to MMWs resulted in an increase of the maximum relative viscosity in lysates. The same effect was observed if the cell lysates were treated with ethidium bromide during lysis (Fig. 7). This antitumour drug is known to be a very specific intercalating agent for DNA. In three independent experiments, ethidium bromide in a concentration of 3–10 μg/ml increased the maximum relative viscosity by 60–80% ($P < 5 \times 10^{-3}$). A concentration dependence obtained had a maximum around a concentration of 5 μg/ml. At this concentration, a maximum relaxation of a nucleoid has been observed under the influence of ethidium bromide [Synzynys et al., 1986]. Ethidium bromide changes the behavior of large DNA-protein complexes due to changes in a conformation of DNA that is supercoiled in cells and cell lysates [Cook and Brazell, 1976]. The data with ethidium bromide provide new evidence of the correlation of AVTD measurements with changes in conformation of the genome structures.

DISCUSSION

In our laboratory, the MMW effect on the GCS of *E. coli* K12 AB1157 cells has been investigated for 8 years. We established the experimental conditions that were critical for reproducibility of the resonance reaction of cells to low-intensity MMWs. The existence of a reproducible and stable effect is a principal reason for studying the mechanisms of the resonance MMW bioaction. Among other things, it is important to know the dependence of resonance frequency and half-width of the resonance on PD. Our

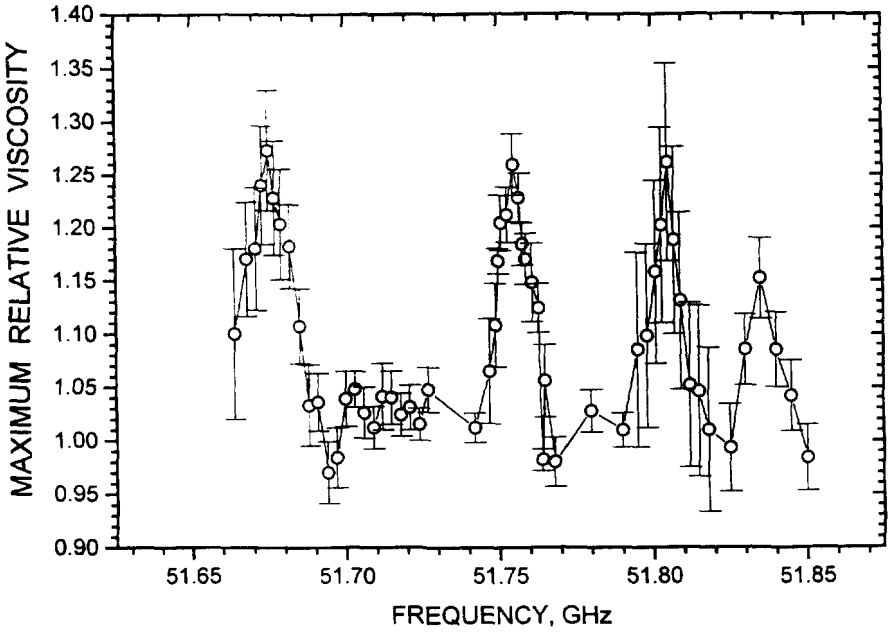

Fig. 5. Frequency dependence of MMW effect at the 10^{-10} W/cm^2 power density. Concentration was 4×10^7 cells/ml during exposure.

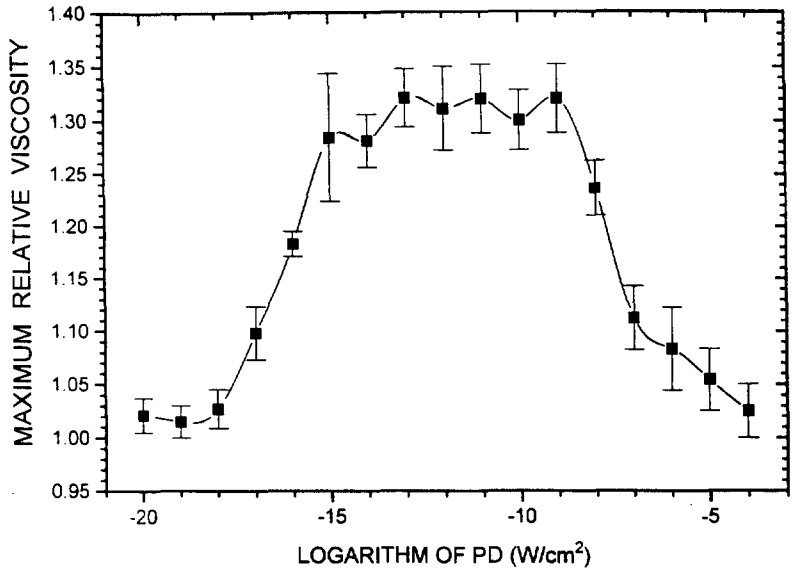

Fig. 6. Power density dependence of maximal relative viscosity in cell lysates after *E. coli* cell exposure to MMWs at the 51.674 GHz frequency. Cell concentration during exposure was 4×10^7 cells/ml.

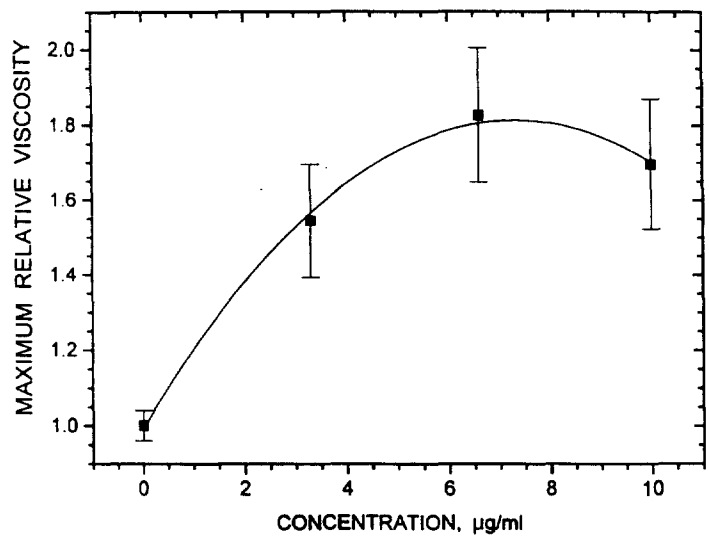

Fig. 7. Dependence of the maximum relative viscosity on the concentration of ethidium bromide during lysis of cells. Ethidium bromide was added at the beginning of lysis.

previous results have revealed the power dependence of the 51.755 GHz resonance half-width [Belyaev et al., 1992d]. Similar findings were reported for yeast cells [Gründler, 1992] and thymocytes of rats [Belyaev and Kravchenko, 1994]. The purpose of the present work was to study this dependence in the broad PD range from 10^{-19} to 3×10^{-3} W/cm^2. In this range, a stable resonance reaction of cells to MMWs with a resonance frequency of 51.755 GHz was registered. The resonance frequency did not change within the accuracy of our measurements. The previous calculations indicated that only a quantum-mechanical approach could be valid at PD < 10^{-15} W/cm^2 [Belyaev et al., 1994a]. This approach to the MMW bioaction was introduced by Frölich [1968] and was developed later in studies by other authors [Didenko et al., 1983; Sitko and Sugakov, 1984; Keilmann, 1986; Arinichev et al., 1993].

The results of the present work could be explained in the framework of the model of electron-conformational interaction (ECI), which has been previously described [Chernavskaya and Chernavskii, 1977; Didenko et al., 1983]. A short description of this model is given below for comparison to experimental data. The ECI model is characterized by three frequency-time parameters: frequency of elastic oscillations, which determines the energy of an electron transition from an initial (|i>) to an excited (|i*>) state (from 10^{10} to 10^{11} Hz); time of electron tunneling from one quasiequilibrium excited state (|i*>) to another one (|f*>; from 10^{-6} to 10^{-7} s); and time of slow electron-conformational change of the macromolecule's ionic frame (from 10^{-2} to 10^{-3} s).

Based on earlier observations, chromosomal DNA can be regarded as a specific cellular target for MMW bioaction, and the DNA parameters can determine an effective MMW frequency and polarization [Belyaev et al., 1992d, 1993b]. It can be assumed that the ionic framework of the DNA-protein complex forms an asymmetric multiplex potential, which is partially presented in Figure 8. In the first stage of interaction, a DNA macromolecule resonantly absorbs a MMW photon, resulting in an electron transition from the initial state, |i>, to the quasiequilibrium state, |i*>. Then, the electron tunnels to another quasiequilibrium state, |f*>. The time of this tunneling determines the half-width of the resonance transition. Electron tunneling to |f*> induces an electron-conformational transition, which involves rearrangement of the ionic frame in a segment of the DNA-protein complex.

The final effect of this electron-conformational interaction is deformation of the macromolecule frame. This conformational rearrangement is caused by migration of electron density along the macromolecule and can be regarded as excitation of long-wave phonons. The system of electron and macromolecule deformation appears to be similar to a polaron. The electron-conformational transition provides a time of from 10^{-2} to 10^{-3} s for changing the ability of the corresponding DNA sequence to bind proteins and metallic ions, which have a significant influence on electron-conformational interaction. If the DNA sequences that control the conformation of the domains of supercoiling are rearranged, then one would expect the GCS changes. The likely candidates for this interaction in *E. coli* cells are repeated extragenic palindromes (REPs), which contain the binding sites for DNA gyrases [Krawiec and Riley, 1990]. These enzymes are located at the attachment sites for supercoiled domains at the cellular membranes and are supposed to control the supercoiling of a nucleoid. It is important that REPs alternate in a left-to-right orientation. These alternations may be responsible for the previously observed difference in the effects of left- and right-handed circularly polarized MMWs at the same resonance frequency [Belyaev et al., 1992b,c].

The frequencies of elastic oscillations in the ECI model are of the same order of magnitude as the MMW frequencies. The lifetime of an electron in the excited state |i*> can be assessed from the resonance half-width. The PD dependence of the 51.755 GHz resonance half-width has a minimum of about 3 MHz at a PD of 10^{-18} W/cm^2 (Fig. 3), wherein the interaction must be assessed by a quantum-mechanical approach [Belyaev et al., 1994a]. A very similar half-width of the resonance of about 3 MHz was obtained previously in investigation of the MMW effect on conformation of hemoglobin in vitro [Didenko et al., 1983]. This effect was also explained in the framework of the ECI model. In the case of a quantum interaction, the lifetime is inversely proportional to the half-width of the energy levels; therefore, it can be estimated as 3×10^{-7} s. This estimate is in good agreement with the time of electron tunneling in the ECI model as well as with the lifetime of excited electrons in solids and semiconductors [Kittel, 1978].

Based on the experimental data, the minimal energy requirement for induction of the GCS changes in *E. coli* cells can be estimated. A statistically significant and reproducible effect was observed when cells at both concentrations were exposed to MMWs with PD = 10^{-18} W/cm^2 for 10 min. Thus, the minimum density of the absorbed energy was determined in our experiments to be at least from 10^{-16} to 10^{-15} J/cm^2. It follows from the ECI model that, during the rearrangement of the macromolecule frame (from 10^{-2} to 10^{-3} s), the system absorbs this minimum energy for 10 min at a PD of about 10^{-13} W/cm^2. At higher PD values, the half-width of the resonance is expected to increase, because it is determined by the overall lifetime of an electron in the |i*> and |f*> levels. Actually, an approximately twofold increase of half-width from 5 to 12 MHz is observed at a PD of about 10^{-13} W/cm^2 (Fig. 3) when exposing the cells at the 4×10^7 cells/ml concentration.

A more dramatic increase of the 51.755 GHz resonance half-width occurred in the range from 10^{-7} to 10^{-4} W/cm^2. A simple explanation of this increase seems to be an additive superposition of the resonances registered in the frequency range of 51.65–51.85 GHz at the lower PD (Fig. 5). However, the results obtained did not support this explanation, because: 1) no fine structure of the frequency dependence was observed for the broadest 51.755 GHz resonances at PD of from 10^{-3} to 10^{-4} W/cm^2 in the region of the resonance frequencies 51.674, 51.805, and 51.835 GHz; and 2) the resonance MMW effect at 51.674 GHz was significant at low intensities and practically disappeared at higher PDs (Fig. 6), whereas the MMW effect should be increased due to superposition of the resonances.

Thus, we assume that a broad resonance is formed by a mechanism other than additive superposition of several sharp resonances. One possibility is that MMW radiation with subthermal intensity induces effective rearrangement of levels in an electron subsystem of DNA. This rearrangement may take place in a magnetic field (Zeeman effect) or in an electric field (Stark effect) of MMW radiation. One cannot exclude the possibility that the effective rearrangement is induced by MMW-induced heating.

Let us note that excited levels li*+> (51.835 GHz) and li*–> (51.674 GHz) are symmetrically situated with respect to the central stable level li*> (51.755 GHz; see Fig. 8). It seems that this fact fits well with the spin models that were suggested previously [Sitko and Sugakov, 1984; Keilmann, 1986]. According to our preliminary data, left-handed circularly polarized MMWs were effective at all three resonances, and right polarization was not effective. In contrast, left-handed polarized MMWs at 10^{-10} W/cm^2 were ineffective at the 51.805 GHz resonance frequency, whereas right polarization affected the cells. The potential dependence of unstable resonance frequencies on static fields is under investigation now.

The present work provides further support for the existence of cooperativity in the resonance reaction of cells to MMWs. The dependence of an EMF effect on the concentration of exposed cells implies an interaction of the cells that is presumably of an electromagnetic nature [Belyaev et al., 1994a]. In the context of the ECI model, secondary radiation is formed during radiative electron transitions in the course of rearrangement of the ionic frame. A possible frequency range of this secondary radiation of the cooperative resonance reaction of cells to weak EMFs was discussed elsewhere [Belyaev et al., 1994a]. The detailed dependence of resonance MMW effect on the concentration of the exposed cells will be described in a separate paper.

Although the resonance effect on the GCS strongly depended on the concentration of exposed cells, the half-width of 51.755 GHz resonance proved to be independent of concentration within a broad range of PD. This data suggests a subcellular structure for resonance interaction of cells with MMWs at a stable frequency. This fact is also in agreement with the previous experimental evidence for the role of DNA as a target of the resonance influence of MMWs on cells [Belyaev et al., 1992d, 1993b]. The possible dependence of half-width at unstable resonance frequencies on the cell concentration during exposure is currently under investigation. The present investigation supports a window dependence of resonance MMW effect on power density in the nonthermal PD range, which has been previously established [Devyatkov, 1973; Gründler et al., 1988].

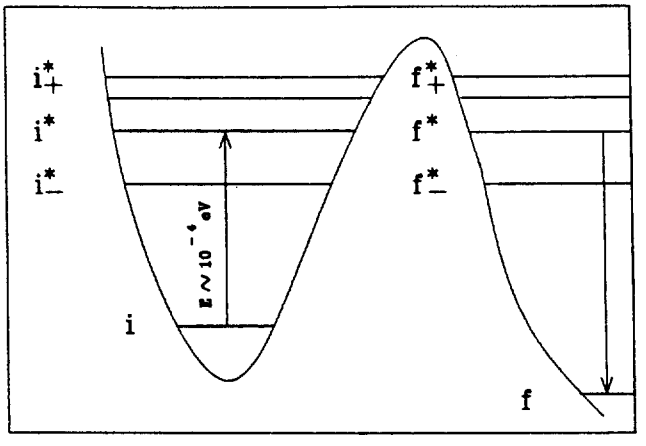

Fig. 8. A part of multiplex asymmetric potential in which the electron-conformational interactions can be directly induced in DNA-protein complex by means of resonance MMW absorption. The vertical axis represents energy, and the horizontal axis represents interparticle distance; neither is drawn to scale. See Discussion text for details.

CONCLUSIONS

The 51.755 GHz resonance effect of MMWs on *E. coli* cells was shown in the range from 10^{-19} to 3×10^{-3} W/cm^2. The half-width of the resonance showed a sigmoid dependence on PD, changing from 3 MHz to 100 MHz. The resonance half-width was the same for different concentrations of exposed cells, whereas the magnitude of the 51.755 GHz resonance effect varied by two to four times. A splitting of the 51.755 GHz resonance into four resonances was observed as the PD decreased from 10^{-4} to 10^{-7} W/cm^2. The PD dependence of the MMW effect at one of these resonance frequencies (51.674 GHz) differed markedly from the corresponding dependence at the 51.755 GHz resonance and had a power window in the range from 10^{-16} to 10^{-8} W/cm^2. The frequency-time parameters of a model of electron-conformational interactions were in agreement with experimental data.

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Kind regards

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E168

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RADIOFREQUENCY ELECTROMAGNETIC FIELDS AND CELLPROLIFERATION

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INTRODUKTION

In spite of numerous studies on the biological effects from exposure to weak electromagnetic(EM) fields, the issue whether continuous exposure to these fields involves health risks, is still a subject for considerable debate. On cellular level effects on cell proliferation, enzyme activities, calcium transport, transcription and chromosome aberations have been reported. On the other hand there are several studies reporting negative results and no effects of exposure to EM fields.

A true mechanism for these observations has not been found yet. The effects seem to be very complex and extremely dependent on experimental conditions, which might have contributed to some of the contradictory results reported. The same may apply to the varying conclusions from epidemiological studies.

We have previously found that exposure to extremely-low-frequency (ELF) magnetic fields resulted in a significant increase in cell growth in human epithelial amnion (AMA) cells[1]. This change in proliferation rate was dependent on exposure times and field densities. A maximum increase in cell proliferation was only found at a specific field density, a socalled "window" and lasted for a definite due to adaptation.

We also showed that superposition of a noise EM field, can inhibit the changes in proliferation rate in AMA cells induced by exposure to ELF magnetic fields[2].

In recent times the use of mobile telephones has accelerated, resulting in an increasing exposure to weak radiofrequency (RF) fields, transmitted from these devices. Consequently the next thing to investigate was, if EM fields generated by microwave radiation, would have an effect on cell proliferation. Though mobile phones are supposed to affect the head primarily and consequently the brain, we could not use primary brain tissue culture, since no cellgrowth takes place here. We decided not to use braintumor cell cultures in the first place, because we wished to compare the effects of microwave radiation under the same conditions as in our previous experiments. Therefore in this case studies were done on the same cell line as the one we used in our previous work.

MATERIALS AND METHODS

Materials and cell lines

Cell proliferation Reagent WST-1 from Boehringer Mannheim Biochemica.

Transformed human epithelial amnion cells (AMA), grown as monolayer cultures in Dulbecco's modified Eagle's medium containing 10% calf serum, penicillin and streptomycin, at 37 °C in 6.0% CO_2 and kept in a Forma Scientific 3164 incubator. All experiments were done on two different clones.

Antibodies to heat-shock proteins Hsp-90 and Hsp-70 from Santa Cruz Biotechnology.

ELF electromagnetic field exposure system

The magnetic fields were generated with the use of Helmholz coils: 12 x 13 cm, 9 cm apart, 2 x 10 turns 1 mm diameter, copper wire. One pair for the AC magnetic field and 3 orthogonal pairs to change the static field. The coils were wound around a square plastic container placed inside the incubator. A low-distortion generator and a power amplifier delivered the signal. The magnetic field was oriented in the plane of the cell plate. Fields were measured with a 3-axis fluxgate meter, Bartington Inst. model MAG-03 MC. The experimental setup has been described before[1].

RF electromagnetic field exposure system

The EM field was generated by signal simulation of the Global System for Mobile communications (GSM) of 960 MHz. The cell cultures, growing in microtiter plates, were exposed in a specially constructed chamber, a Transverse Electromagnetic (TEM) cell. The Specific Absorption Rate (SAR) values for each cell well were calculated for this exposure system[3]. Experiments were performed on AMA cell cultures cells, which were exposed to 960 MHz microwave fields at 3 different power levels in a TEM cell for 20, 30 or 40 min respectively.

Experimental protocol

The cells were grown in microtiter plates, tissue culture grade, 96 wells, flat bottom. Cells were seeded in the 2 central rows, #6 and #7, A-H in 100 µl culture medium. After 24 h field-free incubation at 37°C the cells were growing in their log phase to approximately 40-50% confluency. A plate was then transferred to the exposure chamber i.e. the Helmholtz coil assembly or the TEM cell, in the incubator and exposed to the EM field. Subsequent microtiter plates were exposed to the magnetic field of varying strenght and exposure times. During exposure the field and the temperature in the incubator were monitored continuously.

Proliferation of the cell cultures grown in microtiter plates was determined by a colorimetric assay with quantification in an ELISA plate reader, based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases i viable cells, in the following way : 10 µl WST-1 reagent was added to each well and after 5-6 h incubation at 37 °C in 6.0% CO_2, the absorbance was read. The absorbance was measured at the wavelength of 490 nm and the reference wavelength was 655 nm. The number of proliferating cells was proportional to the absorbance.

For each series of experiments cell proliferation was determined prior to exposure to the field i.e. WST-1 reagent was added to all the wells in row #6 of each plate. After exposure cells were allowed to grow for another 24 h in the field-free incubator. Then cell proliferation was determined in the remaining cells i.e. WST-1 reagent was added to all the wells in row #7 of each plate.

In the control experiments similar cell cultures were grown and incubated in field-free environment and cell proliferation assayed following the same procedure. In the sham exposure experiments the cell cultures were kept for 30 min in the exposure chamber with the field turned off.

Proliferation rate was expressed as the ratio between absorbance after 24 h and absorbance at zero time in percent.

$$Percentproliferationrate = \frac{A_{t=24h}}{A_{t=0}} .100$$

Change in proliferation rate was expressed as the difference between the percent proliferation rate in the exposed culture and the percent proliferation rate in the control experiments.

$$\text{change in proliferation rate} = \text{proliferation rate}_{exposed} - \text{proliferation rate}_{control}$$

Detection of heat-shock proteins

Indirect immunofluorescence was used to detect the presence of heat-shock proteins. Cells were grown on coverslips for 24 h. After exposure to EM fields the coverslips were pre-incubated for 30 min with the antibody in question and then stained by incubation with the secondary antibody, according to standard procedures. The coverslips were then examined by microscopy.

RESULTS AND DISCUSSION

ELF magnetic fields

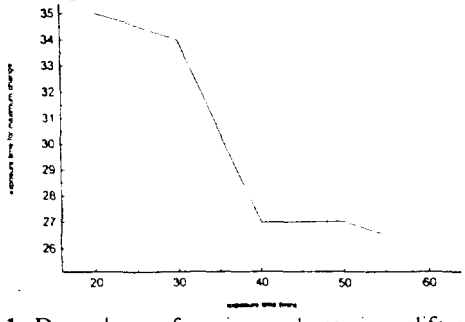

Fig.1. Dependance of maximum change in proliferation rate on exposure time and field strength.

The changes in proliferation rate were determined after exposure to 50 Hz EM fields of different power levels and at varying exposure times. Each result was based on the average mean of 8-9 independent experiments with a 95% confidence interval. We observed in our studies, that the exposure time required to obtain the maximum effect was not the same for the various power levels. It turned out that at low power level the maximum effect was first reached after a longer exposure time than at higher power level. In other words, there is a lineair correlation between the length of exposure time to obtain the maximum effect and the field strength, as shown in Fig.1. This can be explained by assuming "window" effects and adaptation.

RF magnetic fields

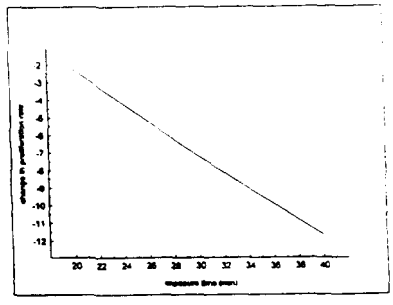

Fig.2a. Change in cell growth after exposure to microwave field. SAR 0.021mW.kg^{-1}

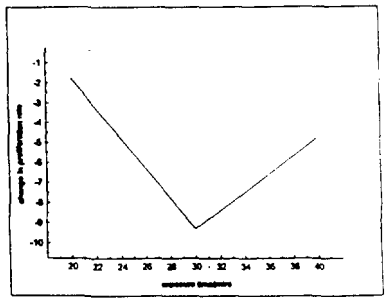

Fig.2b. Change in cell growth after exposure to microwave field. SAR 0.21 mW.kg^{-1}.

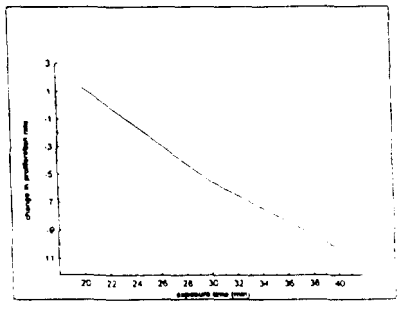

Fig.2c. Change in cell growth after exposure to microwave field. SAR 2.1 mW.kg^{-1}.

Figs.2a-c show the changes in cell growth after exposure to RF electromagnetic fields of varying strength at different exposure times. It was found that cell growth in the exposed cells differed from that in the control and sham exposed cells and a decrease in cell growth was seen. A general lineair correlation between exposure time and growth changes was seen. It also turned out that there was no difference in the changes in cell proliferation for the two clones of cell lines used. As in the case of ELF magnetic fields, maximum cell proliferation during the period following exposure not only varied with the various SAR levels, but also with the length of exposure time (Table 1).

On the other hand repeated periods of exposure did not seem to change the effects.

TABLE 1

Dependance of maximum changes in proliferation rate on exposure time and microwave field strength.

SAR mW.kg-1	Exposure time for maximum change in proliferation rate min
0.021	40
0.21	30
2.1	30

EM fields and the cell cycle

Changes in the cell cycle will affect cell growth. There are two brake-points in the cell cycle through which the cell must pass before it can enter cell division. Progress through each break-point does not only take place in response to exogenous factors, such as growth factors and hormones, but it can also be influenced by the calcium/calmodolin balance, the state of the DNA or by metabolic stress. If there is DNA damage or oxidative stress, the cellular growth regulation may decide on cell death (apoptosis). On the other hand increased cell proliferation is the result of a shorter cell cycle i.e. the first brake-point between the G_1 and S phase is passed at a higher speed.

It has been shown that exposure to EM fields can change the proportion of cells in the G_1 and S phase[4].

Another response to cellular stress is the increased synthesis of certain heat-shock or stress proteins (Hsp). Not only has exposure to EM fields been found to result in an increased transcription of the Hsp gene, but also in rising amounts of certain Hsp, as shown in Table 2. This could not be due to changes in temperature, since at these low field densities temperature changes never exceeded 0.1 °C. Moreover to obtain positive controls temperatures had to be raised 3-5 °C over normal incubation temperature and for a much longer time than in the case of EM fields.

So the mechanism by which electromagnetic fields act could be on the cell cycle level i.e. control of entry into the cell cycle and the cellular policing mechanism to go for either proliferation or apoptosis.

TABLE 2

Presence of heatshock proteins in AMA cell cultures after exposure to EM fields of 50 HZ and varying strength. Exposure time 10 min.

Field strength	**Hsp -90**			**Hsp-70**		
µT	Nu	ER	Golgi	**Nu**	**ER**	**Golgi**
80	+			++	++	
50	+++	+++		++++	++++	
30				++	++	
10				+	+	
control				+	+	

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Data not in paper E168 but supporting results

TABLE

Percent change in proliferation rate in AMA cells after exposure to microwave fields of varying energy levels and different exposure times.

Field strength SAR mW.kg^{-1}	Exposure time min	Change in proliferation rate		
		mean	95% confidence limit	
0.021	20	- 2.2*	- 4.3	8.7
0.021	30	- 7.2*	- 5.5	19.9
0.021	40	- 11.7	1.4	21.9
0.21	20	- 1.75*	- 7.6	11.1
0.21	30	- 9.3	- 0.4	19.1
0.21	40	- 4.8*	- 19.4	28.9
2.1	20	1.3*	- 9.9	12.6
2.1	30	- 5.5	0.17	10.8
2.1	40	- 10.3	5.8	14.8

*not significant P value >0.5 (paired t-test)

Below is the Abstract of E. Czerska et.al. from the following WORKSHOP

"Physical characteristics and possible biological effects of microwaves applied in wireless communication", at the FDA Center for Devices and Radiological Health, Rockville, MD, February 7, 1997; organized by the Bioelectromagnetics Society and hosted by the FDA.

E169

EFFECTS OF RADIOFREQUENCY ELECTROMAGNETIC RADIATION ON CELL PROLIFERATION. Ewa M. Czerska, Jon Casamento, Center for Devices and Radiological Health, Food and Drug Administration, Rockville, Maryland 20857, U.S.A.;
John T. Ning, Indian Health Service, Rockville, Maryland 20857 and Brown University, Providence, Rhode Island 02905, U.S.A.; and Christopher Davis, Electrical Engineering Department,
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Associated with the popularity of wireless communication is the issue of possible biological effects caused by microwave exposure. Human exposure to cellular phone radiation is likely to be localized to part of the head and repetitively applied for various lengths of time. The actual field may vary in frequency and intensity, depending on the type of phone. Some phone types utilize microwaves modulated at extremely low frequencies. This may be the basis for additional concerns because of reports of biological effects from exposure to extremely low frequency electromagnetic fields, including reports (1, 2) of stimulation of cell proliferation in vitro under temperature controlled conditions.

In the present experiment, cells of the human glioblastoma cell line T98G were exposed to 827 MHz frequency modulated radiation, with a waveform identical to that used in digital cellular phones. Each cell culture was exposed in a Crawford cell at either a SAR of 1.6 W/kg (the maximum spatial peak exposure level recommended for the general population in the ANSI C95.1-1991 standard) or at 4.767 W/kg (the maximum exposure level available from our system, which lead to a temperature rise of 1oC). The SAR was determined from temperature/time heating curves produced by exposure to the maximum power available from our system (8.8W forward power). Control cultures were simultaneously placed in a non-activated Crawford cell in the same incubator. Cultures were exposed or sham-exposed for 24 hours continuously. Minimally invasive temperature probes were placed in the tissue culture flasks in both Crawford cells, and temperatures were recorded by computer at 10 min. intervals.

Because of reported associations between cellular phone exposure and the occurrence of a brain tumor, glioblastoma, a human glioblastoma cell line, T98G, derived from a glioblastoma multiforma tumor, was used in ~~line, T98G, derived from a glioblastoma multiforma tumor, was used in~~ this study. The line has an indefinite life span, is anchorage independent, and can be arrested in G1 (before DNA synthesis) when crowded or deprived of serum. The G1 arrest also provides a synchronized cell population in which changes in proliferation are convenient to observe. To start proliferation, G1 cells were trypsynized, resuspended